

## Hidden Genetic Variation in *Agraulis vanillae incarnata* (Nymphalidae)

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**Abstract.** Two culture lines of *Agraulis vanillae incarnata* were established from one wild-collected female. One line was mass selected for reduced black markings, the other for increased black markings. Both lines were maintained through seven generations, at which time the phenotypic differences between the lines diverged in response to selection; a scale deformity also occurred among some individuals in the lightly marked culture. Some genetic aspects of the variation discovered are discussed.

### Introduction

The purpose of the present work was to determine if any genes for albinic, melanic, or immaculate *Agraulis vanillae incarnata* (Riley) were carried by a field collected gravid female. These variants occur rarely in nature, and have been described as aberrants "hewlettii" Gunder (1930), "comstocki" Gunder (1925), "margineapertus" Gunder (1928), and "fumosus" Gunder (1927). The probability of randomly selecting a specimen carrying such gene(s) is admittedly small, and when it became obvious that simple single gene variants were not likely to be expressed in culture, the breeding program was modified to ascertain whether extreme opposite phenotypes could be produced in parallel cultures through mass selection. Since the color and pattern of *A. vanillae* throughout its range in southern California is quite constant, this program would provide information on the amount of hidden genetic variation in the taxon. In *A. vanillae*, which is orange with black markings on the upperside, the approach involved selecting adults toward an all-orange upperside in one culture line and an all-black upperside in the other. A final goal was to cross the selected lines in order to test whether the resulting hybrids would restore the normal phenotype, as might be expected in a complex polygenic system (Lerner, 1954, 1958). Although the original project never reached completion, the results obtained after seven generations are of sufficient interest to be presented here.

## Mating and Rearing Protocol

The original female was collected on 29 October 1982 in Ventura, California. She was confined for oviposition in a flight cage 51 x 51 x 122 cm with several water-potted cuttings of the larval foodplant *Passiflora caerulea*. The cage received afternoon sunshine plus light from a 75-watt GE Gro and Sho Spotlight after sunset. Although courtship and mating in this species occurs throughout the day in warm weather in natural conditions, in this indoor breeding program these activities were limited to the latter half of the day when the flight cage received more light.

Smaller cages for mating single pairs of adults were made from cardboard boxes measuring 23 x 32 x 32 cm. The top and sides were cut out, nylon netting glued over these sides, and a door cut out from the margin of one side. When a specific pair had been chosen and mating required confirmation, the caged butterflies were left in a warm room with ambient light until noon, when they were taken out to a car. When placed on the front seat in sunlight (or occasionally on bright but overcast skies), and with an inside temperature of 24-30°C, matings almost always occurred. Opening one or both car windows to provide a slight breeze helped stimulate mating.

Ovipositing females and their progeny were left in these small cages. Cut *P. caerulea* in water lasts up to two weeks, so new cuttings were added as the older ones began to decline or were consumed, and the larvae eventually found their way onto the new plants. The large flight cage was also used as a rearing cage for broods of up to 800 larvae.

During the last instars it was necessary to clean out the denuded vine stems and frass twice weekly. It was important to keep an adequate supply of foodplants readily available for the larvae, as they cannibalized pupae if these were discovered before foodplant. When most or all larvae had pupated, the cage was cleaned and twigs with prepupae or pupae were cropped to c. 8 cm and pushed into styrofoam mounted on a cardboard base. Eclosing adults were examined for characteristics desired for breeding and placed into appropriate cages. Less extreme phenotypes were saved as papered specimens. All others were liberated.

Under the above conditions, the average time for one life cycle was 45 days. Thus the entire breeding schedule described here required 10½ months.

## The Breeding Program

A pedigree of the breeding program is shown in Figure 1. Except for those instances where a single pair of adults was mated and their offspring reared separately, the majority of the culture lines involved several mixed pairs representing an extreme selected phenotype. Thus the term **mass selection** is used, as multiple individuals were involved in most crosses. The number varied in each generation, but was usually limited to the five

or ten lightest or darkest pairs. When more extreme phenotypes occurred late in the broods, their earlier less extreme counterparts and their ova were discarded or moved to a general mass rearing cage.

Adults from broods 1, 2, 4 and 7 showed no significant variation from typical phenotypes. One-third of the pupae of brood 6 blackened and died, and the remainder discarded. Many adults in brood 5 were unable to fly properly, suggesting viability modifiers. They fluttered upside down on the cage floor and so were not used for breeding, with the exception of a dark female (2(5)DAD) which was mated to a slightly dark male from the mixed brood 2mix. No  $G_3$  or  $G_4$  descendants from this mating expressed the flight affliction. One female (3D/S DAD female #3) from the  $G_3$  of this line showed reduced silver on the hindwing underside and was mated to a male (3D/S D) from the dark culture (results below). The 3D/S D male was mated to two other females.

The hindwing upperside marginal chain pattern tended to break on the discal side in brood BB, a characteristic we call "broken bridges". This line was inbred until the  $G_3$  adults were obtained (Figs. 41-42), then abandoned due to lack of space.

### Extreme Light and Dark Lines

Brood 8 was the major source of lightly marked adults used for selecting the "immaculate" phenotype. This line was maintained through the seventh generation. By the seventh generation, the upperside black spots in the forewing interspaces  $M_3$ ,  $Cu_1$ ,  $Cu_2$  and hindwing interspaces RS,  $M_3$  and at the base of RS- $M_1$ , were entirely absent in most specimens. The forewing marginal triangles and the hindwing marginal chain markings were also greatly reduced. However, the forewing discal cell markings did not respond to selection and remained normal in size. The gradual development of this phenotype is shown in Figures 2-16. The results suggest that different genes or sets of genes independently control these two sets of markings.

A selection of dark adults from the mixed brood was the source of specimens for the dark phenotype. The remainder of specimens from the mixed brood and those from brood 3 were discarded. After four generations they did not exhibit facies as dark as the  $G_2$  dark mixed brood. From this  $G_2$  brood, the darkest specimens (3DD) were bred for one generation. Then, in the  $G_4$ , offspring from the  $G_3$  female 3DD female #2 and  $G_3$  male 3D/S D were included in this brood. The dark line was then inbred until the  $G_7$  adults eclosed. The development of this phenotype is shown in Figures 17-28. (This line shares with the "immaculate" line the  $P_1$  female and  $G_1$  adults in Figures 2-4.)

### Variation in Undersurface Silvering

The 3D/S D male (Fig. 37), which mated three times, displayed reduction in the silver maculation on the hindwing underside. Development of

the silver markings was never a consideration in the selection of the light and dark phenotypes, but the presence of two females with similar reductions in silver markings presented an opportunity to breed this variation. When the female 3D/S D female #1 (Fig. 38) was mated to this male, offspring were as follows: 36 normal males, 52 normal females, one male with slight silver reduction, and three females with moderate silver reduction. When the same male was mated to female 3D/S DAD female #3 (Fig. 40), which also displayed reduced silver markings, the offspring (26 males and 20 females) were all silvered normally. The same male was mated to a normally silvered dark female, 3DD female #2 (Fig. 39), and their offspring (35 males and 49 females) were also all silvered. Finally, from a mixed brood of several pairs of adults with reduced silver markings, the following offspring were obtained: 53 normal males, 58 normal females, and two males and four females with reduced silver markings. The partially unsilvered condition exhibited by several adults was usually not displayed by their offspring, thus the heritability of the character will remain in doubt until further controlled experiments can be performed.

Comparison of the silver markings of the  $G_7$  light phenotypes (Figs. 29-30) with those of the dark phenotypes (Figs. 31-32) show differences in development, especially with the "slipper-shaped" silver spot in interspace RS on the hindwing. In the light phenotype the two halves of this spot nearly coalesce; in the dark phenotype the halves have become widely separated and smaller.

### **Greasy-wing**

A variation having a scale deformity occurred in about 12 individuals of the  $G_5$  generation of the "immaculate" line. These variants were called "greasy-winged" (GW) because of their resemblance to specimens with wings smeared by body fluids. This scale deformity affected all scales, including those on the body. Examples of the deformity are shown together with scales from a normal specimen in the scanning electron microscope photographs in Figures 43-48.

The SEM photographs show that in wild type individuals the pigmented scales differ in shape from silver scales (Fig. 42). At the highest magnifications, the pigmented scales show spaces between the ribs, while the silver scales in the inter-rib area appear solid. In GW individuals all scales are reduced in size and are narrower. Further, the ultrastructure (Figs. 45 & 48) is modified such that the inter-rib area of all scale types appears partially filled or plugged. The effect is apparently a breakdown of the diffractive properties of the scale surface, producing partial transparency.

The source of GW variants was the pooled brood of 4imm adults. This brood consisted of ca. 10-15 pairs of normal adults, and their pooled  $G_5$  offspring consisted of ca. 100 normal "immaculate" phenotypes and ca. 12 GW adults (the 5GW and 6GW lines were established from these). None of



these "greasy-wings" was as extreme as those which occurred in later broods. However, extreme "greasy-wings" did result from matings between normal "immaculate"  $G_6$  adults.

The results of further crosses of the 4imm line to show inheritance of GW follow:

<b><math>G_4</math> offspring</b>	100 normal:12 GW	
	<b>Normal x Normal</b>	<b>GW x GW</b>
	<b>(pair)</b>	<b>(mass mating)</b>
<b><math>G_5</math></b>		
Normal male	12	2
Normal female	10	0
GW male	9	64
GW female	7	66
	<b>From above,</b>	
	<b>Normal x Normal</b>	
	<b>(pair)</b>	
<b><math>G_6</math></b>		
Normal male	19	
Normal female	23	
GW male	17	
GW female	7	

Extreme GW examples are illustrated in Figures 33 & 36. The transparency of the wings is indicated by the striped paper placed under the wings of the specimen in Figure 34.

Data from both  $G_5$  and  $G_6$  pairings indicate the character is autosomal and probably digenic, resulting from the interaction of paired non-linked complementary genes. Thus a cross of two heterozygote wild types would be expected to produce a 9+:7 GW ratio. The pooled data give a better fit ( $X^2 = 1.4$ ,  $df = 1$ ,  $p > .25$ ) to 9:7 than to the 3:1 ratio for a single recessive ( $X^2 = 10.1$ ,  $df = 1$ ,  $p < .001$ ). Further, if GW were due to a simple recessive, the phenotype should have appeared in the  $G_2$  or  $G_3$  generations.

The "greasy-wing" scale deformity probably decreases fitness, and would appear to be strongly selected against in nature. Meconium discharged from eclosing adults was not repelled by individuals with the GW wings, but rather stuck to them and dried, sometimes causing wings to stick together. Other specimens had lesions on the wings which oozed body fluids at the time of wing expansion. Individuals that were able to expand their wings successfully behaved normally; but because of the non-repellent nature of their wings, they would most likely experience difficulty in humid or rainy conditions.

The cultures were terminated at the end of August 1983 for two reasons. First, the abandoned orchard which had become completely overgrown with *Passiflora caerulea* was cleared for development, eliminating the foodplant resource, and an artificial diet was not further available. Second, TED developed a bronchial irritation from constant exposure to the culture, which was maintained in his living quarters.

## Discussion

The exploratory work reported here clearly shows that a significant amount of potential genetic variation was present but masked in a single mated female of a phenotypically constant butterfly. The variants produced during the course of seven generations of inbreeding and selection included individuals with substantially greater or lesser quantities of melanin than typical *A. vanillae*, individuals with the scale anomaly greasy-wings, and individuals with behavioral modifications. The genetic systems producing these effects range from what appears to be complexes of polygenes controlling general wing pattern density to a digenic mendelian pair of genes for greasy-wings. Little research on inbreeding and mass selection has been reported in the Lepidoptera (Robinson, 1971), although inbreeding and selection is a widely used technique to determine the amount of genetic variation in organisms (Lerner, 1954, 1958; Lewontin, 1974). However, note Oliver's, 1981, work on inbreeding depression and some early work by Schrader, 1911. Oliver (1981) showed genetic variability in terms of lethal equivalents in 12 lepidopteran species.

To the extent that the mated female tested is representative (a subsequent abbreviated three generation experiment involving another female produced both light and dark trending individuals, as well as the "broken bridges" phenotypes) one might ask: why are such variants not observed more frequently in nature? Two named aberrants "comstocki" and "margineapertus" occasionally turn up in collections. These aberrations closely resemble the extreme dark and light individuals selected in this breeding program. Our results imply the genetic control of wing melanin is not based on a few simple mendelian genes at the simultaneous recovery of both "comstocki" and "margineapertus" types from a single individual would be quite unlikely given the rarity of these aberrants in nature. An albino aberrant "hewlettii" occurs very rarely in nature. The "greasy-wing" trait, reported above, has not been reported previously. The data reported here lead us to conclude that substantial heterozygosity for wing character variants exist in natural populations of *A. vanillae*.

The magnitude of genetic variation we extracted from a single mated individual has implications to both conservation and systematics. In conservation the increasing use of captive breeding programs cannot overemphasize the necessity of attempting to utilize a selective and breeding scheme which maintains wild type individuals, while sequestering variance. In systematics, the results are interesting as applied to butterflies, since butterfly taxonomy at both species and subspecies levels is based largely on wing character states which involve minor changes. The range of variants mass selected from our single female could well represent several different subspecies, if not species, if found fixed in natural populations.

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### Literature Cited

- COMSTOCK, J. A., 1927. *Butterflies of California*, publ. by author. Los Angeles. 334 pp.
- GUNDER, J. D., 1925. Several new varieties of and aberrant Lepidoptera from California. *Ento. News* 36:5-6.
- \_\_\_\_\_, 1927. New transition form or "abs" and their classification. *Ento. News* 38:129-138.
- \_\_\_\_\_, 1928. Additional transition forms. *Can. Ento.* 60:162-168.
- \_\_\_\_\_, 1928b. New butterflies and sundry notes. *Bull. Brooklyn Ento. Soc.* 24:327-328.
- LERNER, I. M., 1954. *Genetic homeostasis*. Wiley, New York. 134 pp.
- \_\_\_\_\_, 1958. *The genetic basis of selection*. Wiley, New York. 298 pp.
- LEWONTIN, R. C., 1974. *The genetic basis of evolutionary change*. Columbia University Press, New York. 346 pp.
- OLIVER, C. G., 1981. A preliminary investigation of embryonic inbreeding depression in twelve species of Lepidoptera. *Jr. Lep. Soc.* 35:51-60.
- ROBINSON, R., 1971. *Lepidoptera genetics*. Pergamon, Oxford. 687 pp.
- SCHRADER, W., 1911. Inbreeding of *Junonia coenia* under high temperatures through twenty-two successive generations. *Pomona Jr. Zool. & Ento.* 4: 673-677.

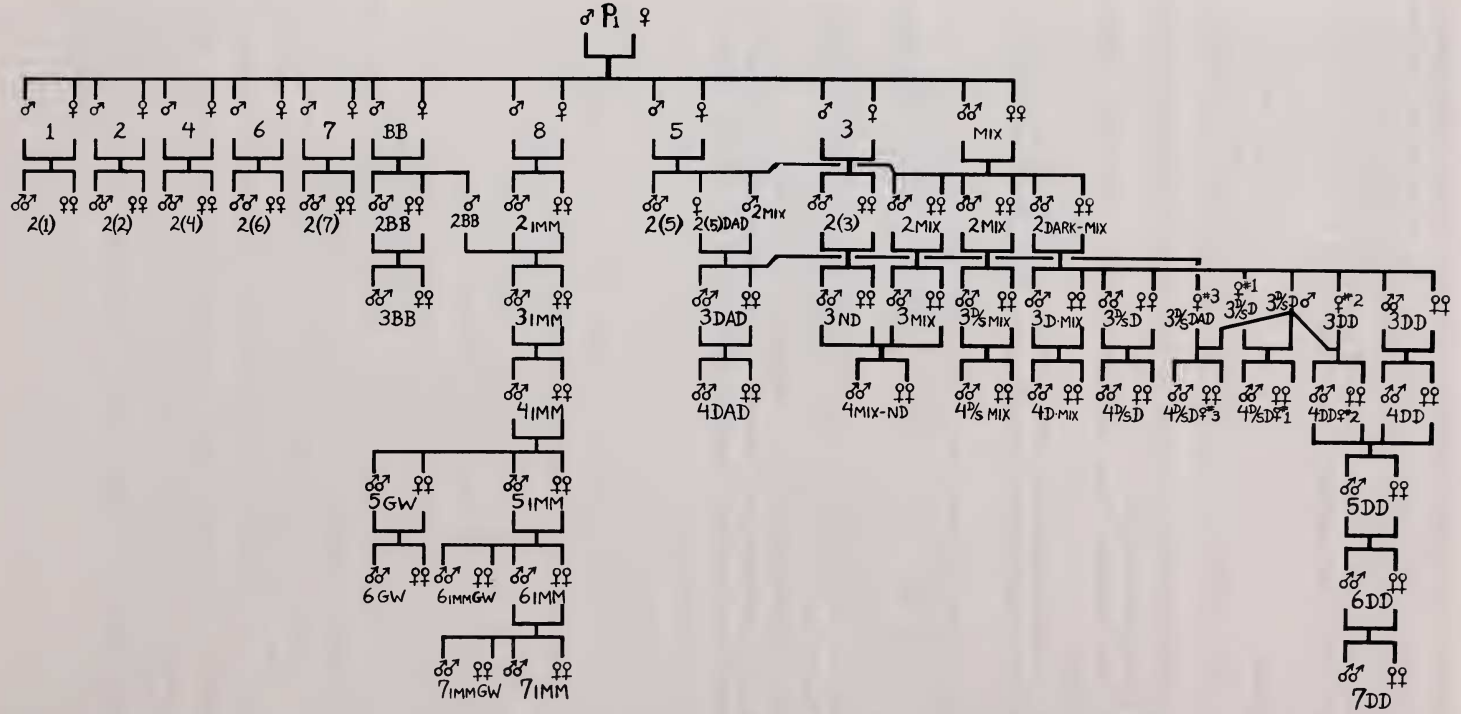
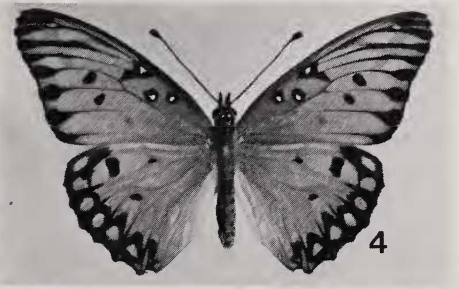
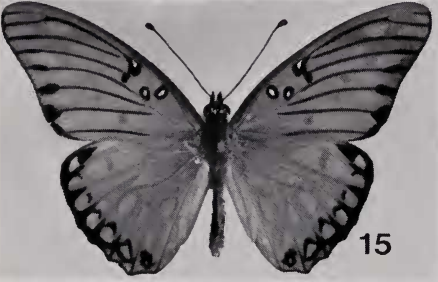


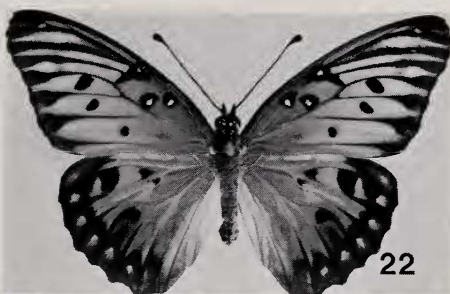
Fig. 1. Pedigree of mass selection breeding project using *Agraulis vanillae incarnata*. Single sex symbols indicate one pair of adults were mated to obtain progeny; double sex symbols indicate two or more pairs of adults. Numbers indicate filial generation. Abbreviations : BB Broken Bridges; D Dark; DAD Dark and Disabled; DD Darkest Darks; D/S Diminished Silver; GW Greasy-winged; IMM immaculate phenotype; MIX Large number of randomly mated individuals; ND Next Darkest.

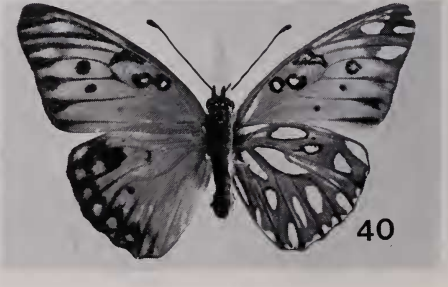
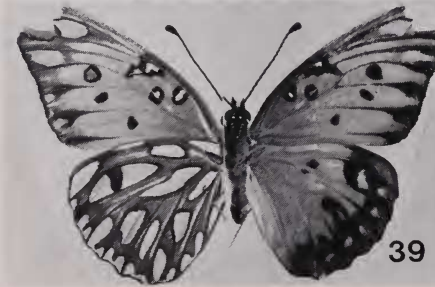
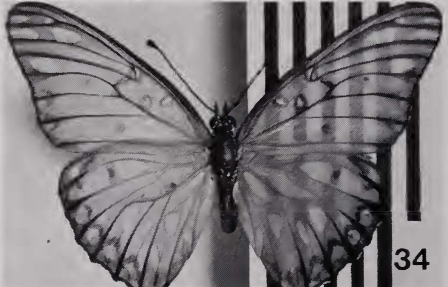




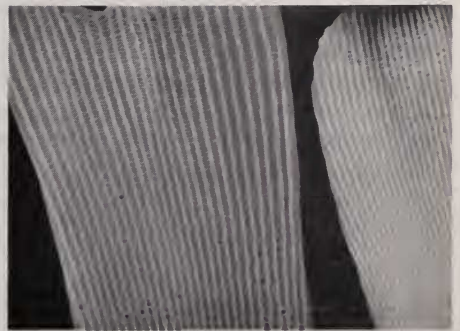
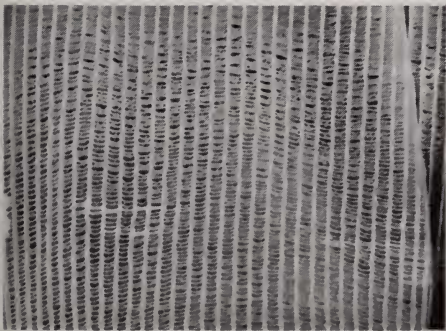
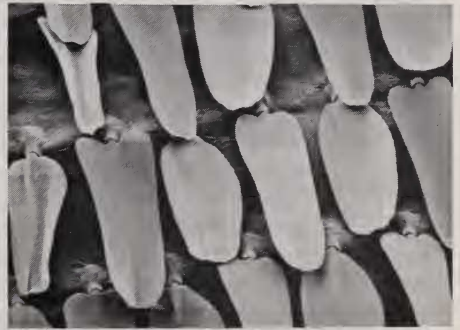
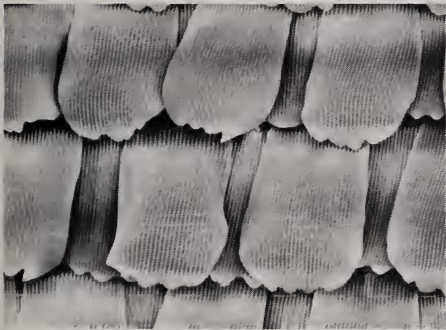
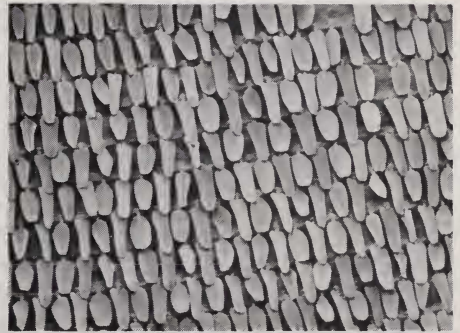
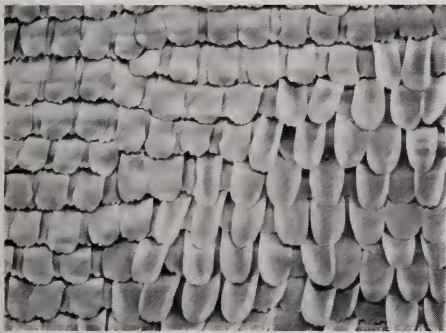












Selectively bred adults of *Agraulis vanillae incarnata*. Males on left, females on right, except where noted otherwise.

Fig. 2. wild P<sub>1</sub> female.

Figs. 3, 4. G<sub>1</sub>.

Adults bred for reduced black markings ("immaculate" phenotype):

Figs. 5, 6: G<sub>2</sub>;

Figs. 7, 8: G<sub>3</sub>;

Figs. 9, 10: G<sub>4</sub>;

Figs. 11, 12: G<sub>5</sub>;

Figs. 13, 14: G<sub>6</sub>;

Figs. 15, 16: G<sub>7</sub>.

Adults bred for increased black markings (dark phenotype):

Figs. 17, 18: G<sub>2</sub>;

Figs. 19, 20: G<sub>2</sub>;

Figs. 21, 22: G<sub>4</sub>;

Figs. 23, 24: G<sub>5</sub>;

Figs. 25, 26: G<sub>6</sub>;

Figs. 27, 28: G<sub>7</sub>.

Figs. 29, 30: Undersides of "immaculate" phenotypes in Figs. 15 and 16.

Figs. 31, 32: Undersides of dark phenotypes in Figs. 27 and 28.

Figs. 33, 34: "Greasy-winged" scale deformity. Male F<sub>6</sub>, female F<sub>7</sub>.

Figs. 35, 36: Undersides of Figs. 33 and 34.

Fig. 37. G<sub>3</sub> male 3D/S. Left side ventral.

Fig. 38. G<sub>3</sub> female 3D/S D♀#1. Left side ventral.

Fig. 39. G<sub>3</sub> female 3DD♀#2. Left side ventral.

Fig. 40. G<sub>3</sub> female 3D/S D♀#3. Right side ventral.

Figs. 41, 42. G<sub>3</sub> "broken bridges" phenotype.

Fig. 43. Scanning electron microscope photograph of the wing underside of a normal female, brood 7imm. Magnification 160 X. Dark brown scales at upper left, silver scales at lower right.

Fig. 44. Same as Fig. 43, magnification 640 X, dark brown scales.

Fig. 45. Same as Fig. 43, magnification 2500 X.

Fig. 46. Wing underside of a "greasy-winged" female, brood 7immGW. Magnification 160 X. Dark brown scales.

Fig. 47. Same as Fig. 46, magnification 640 X.

Fig. 48. Same as Fig. 46, magnification 2500 X.

Note: The photographs of the adult butterflies were all shot at f5.6 at 1/250th of a second exposure. Varying developing exposures by the automated commercial processing equipment has resulted in photographs in which the orange ground color of the butterflies appears to vary in brightness, which in reality is not the case. Both males and females of the "immaculate" phenotype are consistently bright orange. Dark phenotype males are slightly deeper orange, and their females are auburn-orange.